Remarks

Applicants have amended claims 2 and 30. With the entry of this amendment, claims 14, 15, 30 and 45-50 are pending and claims 1-13, 16-29, and 31-44 are canceled.

Rejections under 35 USC § 112

Applicants traverse the rejections under Section 112 for the following reasons.

The Federal Circuit has held that "a considerable amount of experimentation is permissible, if it is merely routine, **or** if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed." *In re Wands*, 858 F.2d 731, 737, 8 USPQ.2d 1400, 1404 (1988). (Emphasis added.) Applicants contend that in light of the specification and state of the art, using the present compounds for the present claims would be a matter of routine optimization, achievable by one of ordinary skill in the art without undue experimentation. As discussed below, the present application has many similar facts to *Wands*.

First, the level of one of ordinary skill in the art is high. For example, the artisan of ordinary skill who would generally practice the invention would likely be a Ph.D. and/or M.D. specializing in pharmacology. An artisan of such a high skill level would easily be capable of testing the compounds against any of the claimed disorders or biological functions and optimizing the appropriate dosage level. The skilled artisan, in light of the specification, would have sufficient knowledge to practice the invention at the time of filing.

Second, the specification provides guidance on how to use the invention. Pages 120-126 provide guidance on the therapeutic use of the invention. Based on the guidance provided in the specification, one of skill in the art could have assayed the compounds of the present invention in known *in vivo* or *in vitro* models for the disorders of the present claims. The relative routine nature of such testing is supported by the attached article, by Zhu *et al.*, Bioorganic & Medicinal Chemistry Letters 12 (2002) 403-406.

Third, the Examiner has misconstrued the level of unpredictability in the art, which does not rise to the level of requiring undue experimentation. The Huirne and Lambalk Lancet reference cited by the examiner actually supports that one of skill in the art could have been practiced the claimed methods. As noted in this reference "GnRH-receptor antagonists, that immediately block GnRH's effects, [have recently] been developed for clinical use with acceptable pharmacokinetic, safety and commercial profiles." The authors continue that "[a]ll current indications for Gn-RH-agonists desensitizations may prove to be indications a GnRH-receptor antagonist, including endometriosis, leiomyoma, and breast cancer in women, benign prostatic hypertrophy and prostatic carcinoma in men, and central precocious puberty in children."

The fact that the "best *clinical evidence* so far has been in assisted reproduction and prostate cancer" does not mean the present invention is not enabled. (Emphasis added.) As noted in the below summary from MPEP § 2107.02 IV, the need for clinical trails and data is regulatory and separate from the 35 USC § 112 statutory requirement of the USPTO.

> Office personnel should not impose on applicants the unnecessary burden of providing evidence from human clinical trials. There is no decisional law that requires an applicant to provide data from human clinical trials to establish utility for an invention related to treatment of human disorders (see *In re Isaacs*, 347 F.2d 889, 146 USPQ 193 (CCPA 1963); *In re Langer*, 503 F.2d 1380, 183 USPQ 288 (CCPA 1974)), even with respect to situations where no art-recognized animal models existed for the human disease encompassed by the claims. *Ex parte Balzarini*, 21 USPQ2d 1892 (Bd. Pat. App. & Inter. 1991) (human clinical data is not required to demonstrate the utility of the claimed invention, even though those skilled in the art might not accept other evidence to establish the efficacy of the claimed therapeutic compositions and the operativeness of the claimed methods of treating humans).

Rejections under 35 USC § 103

Applicants traverse the prior art rejections of record and assert that the claimed invention is non-obvious for the following reasons. Applicants contend that the Examiner has not established a *prima facie* case of obviousness, which requires (1) a teaching or suggestion of all the elements of the claims in the cited references, (2) motivation to combine or modify references to arrive at the present invention, and (3) a reasonable expectation of success of the

invention.

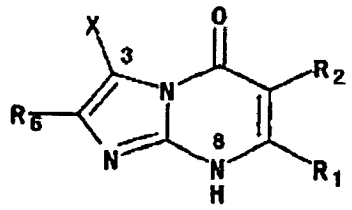
The Ikesu patent relates to compounds for color photographic material, which is not related to pharmaceutical activity. Applicants contend that whatever equivalency of the compounds of Ikesu in field of color photographic material is simply not applicable to antagonizing GnRH. Therefore, the Ikesu reference cannot provide any reasonable expectation of success that the compounds of Ikesu could have been used in any pharmaceutical application, much less for antagonizing GnRH.

The Abdalla reference relates to compounds that are useful for killing microbes. Applicants contend that whatever equivalency of the compounds of Abdalla in field of microbial agents is simply not applicable to antagonizing GnRH. Therefore, the Abdalla reference cannot provide any reasonable expectation of success that the compounds of Abdalla could have been used for antagonizing GnRH.

Moreover, the Examiner has not established why one of skill in the art would be motivated to develop compounds for antagonizing GnRH from references in field of color photographic material or related to the treatment of microbial agents. Therefore, applicants contend that the Examiner has failed to establish the requisite motivation.

Applicants also contend that the present claims are patentable over the cited references for the reasons given below, which were presented in the previous Response.

Ikesu et al. (USP5,208,141) discloses a compound having the following structural formula:



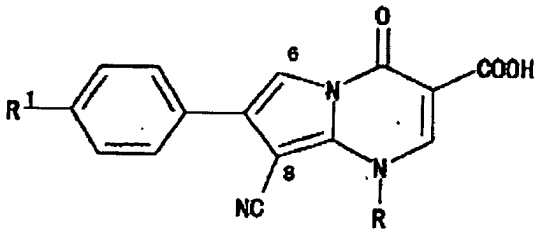
wherein X is a hydrogen atom or a substituent capable of slitting off upon reaction with an oxidation product of a color developing agent (halogen atom, alkoxy, aryloxy, heterocyclic oxy, acyloxy, sulfonyloxy, alkoxycarbonyloxy, aryloxycarbonyl, alkyloxalyloxy, alkoxyoxalyloxy, alkylthio, arylthio, heterocyclic thio, alkyloxythiocarbonylthio, acylamino, sulfonamide, nitrogero-containing heterocycle linked via a nitrogen atom, alkyloxycarbonylamino, aryloxycarbonylamino and carboxyl group).

However, in the sole compound actually disclosed in Ikesu et al., X is hydrogen atom, halogen, aryloxy, arylthio or nitrogen-containing heterocycle linked via a nitrogen atom, the carbon atom at the 3-position of the oxoimidazo[1,2-a]pyrimidine, which has N-containing 5-membered ring, and X form a C-H bond, C-Hal (halogen) bond, C-O bond, C-S bond or C-N bond, and the 8-position of the oxoimidazo[1,2-a]pyrimidine is unsubstituted.

In addition, the use of the compound of Ikesu et al. is also for a silver halide color photographic light-sensitive material, which is a non-pharmaceutical use.

In contrast, the present invention relates to a compound characterized in that it has a substituent at the 1-position of oxopyrrolo[1,2-a]pyrimidine (corresponding to the 8-position of oxoilmidazo[1,2-a]pyrimidine), which has a 5-membered ring free of N, and a substituent that forms a C-C bond with the carbon atom at the 6-position of oxopyrrolo[1,2-a]pyrimidine (corresponding to the 3-position of oxoimidazo[1,2-a]pyrimidine). Thus, the compound of Ikesu et al. and the compound of the present invention are completely different in chemical structure and nothing in Ikesu or Abdalla would suggest the compounds of the present claims. In addition, the present invention relates to a pharmaceutical use, particularly the use as a GnRH antagonist, which is completely different from the use of the compound of Ikesu et al.

Abdalla et et al. (J. Heterocyclic Chemistry 24, 297, 1987) discloses a compound having the following structural formula:



Namely, Abdalla et al. merely disclose a compound wherein the 8-position of oxopyrrolo[1,2-a]pyrimidine is substituted by CN and the 6-position of oxopyrrolo[1,2a]pyrimidine is not substituted.

Moreover, the use of the compound of Abdalla et al. is an antimicrobial agent.

In contrast the present invention relates to a compound characterized in that has a substituent at the 6-position of oxopyrrolo[1,2-a]pyrimidine and the 8-position of oxopyrrolo[1,2-a]pyrimidine is unsubstituted. Therefore, the compound of Abdalla et al. and

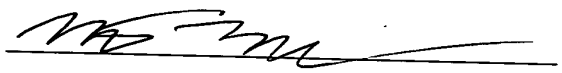
the compound of the present invention are completely different in chemical structure. In addition, the use of the compound of the present invention is a GnRH antagonist, which is completely different from the use of the compound of Abdalla et al.

As discussed above, the compounds of Ikesu et al. and Abdalla et al. and the compounds of the present invention are completely different in chemical structure and use.

Conclusion

Applicants submit that the present application is now in condition for allowance, and favorable reconsideration thereof is respectfully requested. If the Examiner believes that an interview would advance prosecution of the application, he is invited to contact the undersigned by telephone. If there are any unaccounted fees due in connection with the filing of this Amendment, please charge the fees to our Deposit Account No. 19-0741.

Respectfully submitted,



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Should additional fees be necessary in connection with the filing of this paper, or if a petition for extension of time is required for timely acceptance of same, the Commissioner is hereby authorized to charge Deposit Account No. 19-0741 for any such fees; and applicant(s) hereby petition for any needed extension of time.

Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 403–406

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

A Novel Synthesis of 2-Arylpyrrolo[1,2-*a*]pyrimid-7-ones and Their Structure–Activity Relationships as Potent GnRH Receptor Antagonists

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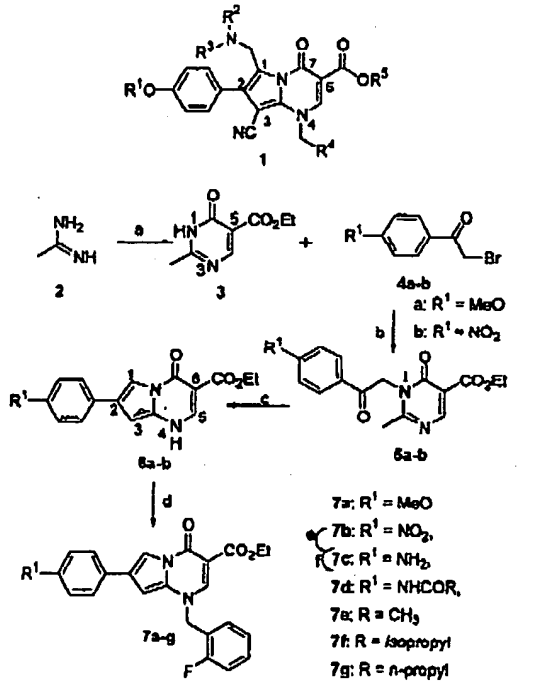
Abstract—In the process of developing GnRH receptor antagonists, a novel base-catalyzed cyclization of compounds 5a–b was discovered, which led to the formation of the 2-aryl pyrrolo[1,2-*a*]pyrimid-7-one core stuctures 6a–b. These intermediates were further modified at positions 1, 2, 4 and 6 to afford a series of potent GnRH antagonists with low nanomolar K_i values. © 2002 Elsevier Science Ltd. All rights reserved.

In the previous letter,[1] we discussed the initial SAR study of a novel series of 1-aminomethyl-2-aryl-3-cyano-pyrrolo[1,2-*a*]pyrimid-7-ones (1) as human gonadotropin-releasing hormone (hGnRH) receptor antagonists. Here, we report a novel synthesis of the bicyclic 2-arylpyrrolo[1,2-*a*]pyrmid-7-one core structure as well as further SAR studies of its derivatives as potent hGnRH receptor antagonists.

The novel synthesis of 2-arylpyrrolo[1,2-*a*]pyrimid-7-ones, represented by 6a–b, is outlined in Scheme 1. Amidine 2 was refluxed with diethyl ethoxymethylene malonate in the presence of EtONa in EtOH[2] to afford the corresponding pyrimidone 3. Compound 3 was then treated separately with α-bromoacetophones 4a and 4b in the presence of tetrabutylammonium fluoride (TBAF) in THF to give pyrimidones 5a and 5b, respectively. The regioselective N^1-alkylation was confirmed by NOE experiments. Catalyzed by NaH in THF, pyrimidones 5a–b underwent intramolecular cyclization to give the bicyclic core structures 6a–b in good yields. Intermediates 6a–b were then exposed to 2-fluorobenzyl bromide and 1 M TBAF in THF to form compounds 7a–b. Compound 7b was further reduced by hydrogenation over Pd/C to yield the corresponding aniline 7c. Subsequent acylation with carboxylic anhydrides gave the amides 7d (7e–g).



Scheme 1. Reagents and conditions: (a) diethyl ethoxymethylene malonate, EtONa/EtOH reflux; (b) TBAF, THF; (c) NaH, THF; (d) TBAF/THF, 2-fluorobenzyl bromide; (e) H_2, Pd/C, HOAc, 30 psi; (f) $(RCO)_2O$, Et_3N, THF.

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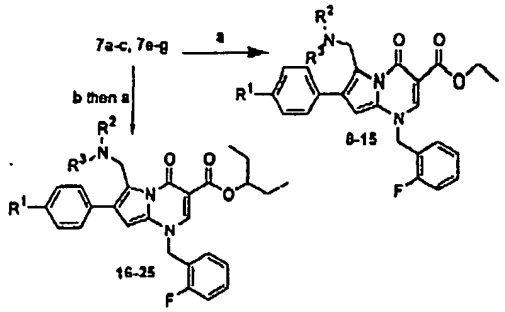
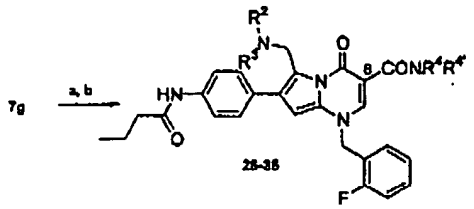
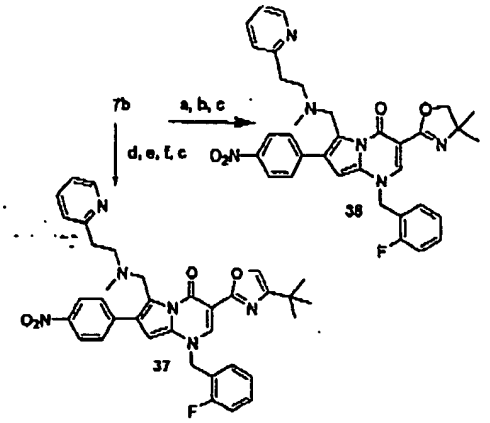


Scheme 2. Reagents and conditions: (a) R^2R^3NH, CH_2O in water, EtOH; (b) 3-pentanol, BuLi, THF.



Scheme 3. Reagents and conditions: (a) $R^4R^{4'}NH$, Et_2Al, DCE, reflux; (b) R^2R^3NH, CH_2O in water.

As illustrated in Scheme 2, advanced intermediates 7a–c and 7e–g were diversely modified at position 1 via a simple Mannich reaction[3] with various amines in the presence of formaldehyde. The structures of a selected series of final compounds 8–15 are presented in Table 1. In addition, modifications of the 6-ethoxy-carbonyl group on 7a–c and 7e–g were also explored (Scheme 2) and the ethyl esters were *trans*-esterified to the corresponding 3-pentyl esters by lithium 3-pentoxide, formed in situ from butyl lithium and 3-pentanol in THF, followed by Mannich reactions to afford the amines 16–25 as shown in Table 2. Furthermore, ethyl ester 7g was



Scheme 4. Reagents and conditions: (a) NaH, 2-amino-2-methyl-propanol, THF; (b) $SOCl_2$, THF; (c) 2-(2-methylaminoethyl)pyridine, CH_2O in water; (d) LiOH, THF/MeOH/ H_2O; (e) K_2CO_3, 1-bromopinacolone, DMF; (f) NH_4OAc/HOAc, 100 °C.

converted to a variety of carboxamides by reaction with pre-formed triethyl aluminum and amine ($R^4R^{4'}NH$) complexes in 1,2-dichloroethane at 90 °C, followed by Mannich reactions to give products 26–35 as depicted in Scheme 3.

Scheme 4 shows the synthesis of two heterocyclic derivatives (36 and 37) of the 6-carboxylic ester. Reaction of 7b with pre-mixed NaH and 2-amino-2-methyl-propanol solution in THF, followed by treatment with thionyl chloride formed an oxazoline,[4] which was then subjected to Mannich conditions to yield 36. Compound 7b was hydrolyzed by LiOH in a mixture of THF, MeOH and water and the resulting acid was treated with 1-bromopinacolone in the presence of K_2CO_3, followed by reflux in HOAc with NH_4OAc to produce an oxazole.[5] Mannich reaction yielded the desired compound 37.

Table 1. Binding affinities of compounds 8–15 on the hGnRH receptor[7]

Compd	R^1	R^2R^3NH	K_i (nM) human
8	MeO	BnNHMe	400
9	MeO	(2-pyr)-$(CH_2)_2$NHMe	55
10	NO_2	BnNHMe	450
11	NH_2	BnNHMe	190
12	MeCONH	BnNHMe	42
13	*i*-PrCONH	BnNHMe	44
14	*i*-PrCONH	(2-pyr)-$(CH_2)_2$NHMe	14
15	*n*-PrCONH	(2-pyr)-$(CH_2)_2$NHMe	1.2

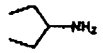
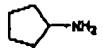
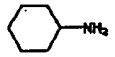
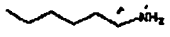
Table 2. Binding affinities of compounds 16–25 on the hGnRH receptor[7]

Compd	R^1	R^2R^3NH	K_i (nM) human
16	MeO	BnNHMe	62
17	MeO	(2-pyr)-$(CH_2)_2$NHMe	14
18	NH_2	BnNHMe	100
19	MeCONH	BnNHMe	4.0
20	MeCONH	(2-pyr)-$(CH_2)_2$NHMe	2.8
21	*n*-PrCONH	BnNHMe	3.8
22	*n*-PrCONH	(2-pyr)-$(CH_2)_2$NHMe	2.7
23	*n*-PrCONH	Me_2NH	130
24	*n*-PrCONH	$Et_2N(CH_2)_2NH$-Me	5.8
25	*n*-PrCONH	$PhCH_2)_2NHMe$	2.1

All of the synthesized compounds were evaluated for their ability to compete for des-Gly^{10}[^{125}I-Tyr,^{5}DLeu,^{6}NMeLeu,^{7}Pro^{9}-NEt]GnRH radioligand binding to the cloned human receptor.[6] The binding assay results on varying the R^1 group with either *N*-methyl-benzylamine or *N*-methyl-*N*-(2-pyridyl)ethylamine as R^2R^3NH are presented in Table 1.[7] Compounds **8** and **9** were substantially more potent than their corresponding 3-cyano analogues.[1] These data suggest that position 3 of the bicyclic core prefers not to be substituted. Replacement of the electron donating methoxy group of R^1 with a strong electron withdrawing nitro group had no substantial impact on potency (**10**, 450 nM). However, reduction of the nitro compound to the corresponding amino analogue **11** yielded a 2-fold improvement in the binding affinity. A hydrogen bond acceptor together with a lipophilic group seems to be the preferred R^1 group as the acylated analogues **12** and **13** had 5-fold improvement in potency, in comparison with the anilino compound **11**. Furthermore, a 3-fold enhancement of potency was obtained by use of *N*-methyl-*N*-2-(2-pyridyl)ethylamine as R^2R^3NH (**14**, K_i = 14 nM). Surprisingly, a slight change from the branched *iso*-butyrylamino group (*i*-PrCONH) to a linear *n*-butyrylamino group (*n*-PrCONH) of R^1 provided a 10-fold increase in the binding affinity (**15** vs **14**).

Table 2 lists the binding affinity data of compounds **16**–**25**, in which all molecules contain the 3-pentyl carboxylate instead of the ethyl carboxylate at position 6. A direct comparison between compounds **16**, **17**, **18** and compounds **8**, **9**, **11** reveals that the 3-pentyl carboxylate significantly enhanced the binding affinity. Consequently, combination of 3-pentyl carboxylate and acetamido groups in compound **19** gave a K_i value of 4 nM, while its corresponding ethyl carboxylate **12** was 10-fold less potent. Interestingly, the binding enhancement by incorporation of the *N*-methyl-*N*-2-(2-pyridyl)ethylamine at the 1-methyl position in the ethyl carboxylates was no longer observed here, as compound **20** was only slightly more potent than compound **19**. This trend was also observed between compounds **21** and **22**. Further evaluation of different substituents on the basic amine revealed that the simple dimethylamino analogue **23** had respectable potency (K_i = 130 nM). A non-aromatic side chain containing a basic amine (**24**) was only 2-fold less potent than compound **22**. The binding affinity of **25** provided more evidence that the *N*-methyl-*N*-2-(2-pyridyl)ethylamine was no longer crucial for high potency, since phenethyl replacement of the 2-pyridylethyl group as the substituent on the basic amine side chain gave very similar potency. It was worth noting that such replacement led to complete loss of binding affinity in the previous SAR study on compound **1**.[1]

Table 3. Binding affinities of compounds 26–35 on the hGnRH receptor[7]

Compd	R_2R_3NH	$R_4R_4'NH$	K_i (nM) human
26	(2-pyr)-$(CH_2)_2$NHMe		21
27	(2-pyr)-$(CH_2)_2$NHMe		9.2
28	(2-pyr)-$(CH_2)_2$NHMe		10
29	(2-pyr)-$(CH_2)_2$NHMe		32
30	(2-pyr)-$(CH_2)_2$NHMe		3.3
31	(2-pyr)-$(CH_2)_2$NHMe		630
32	(2-pyr)-$(CH_2)_2$NHMe		1.1
33	BnNHMe		17
34	BnNHMe		154
35	$Et_2N(CH_2)_2NH$–Me		440

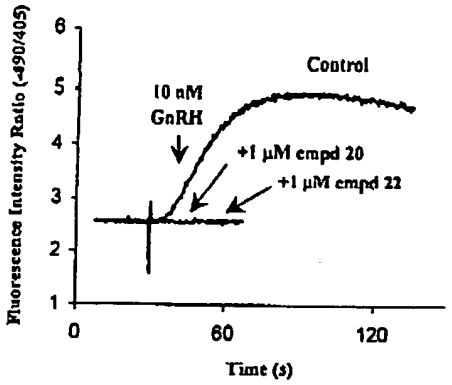


Figure 1. Inhibition of GnRH stimulated Ca^{++} flux by compounds 20 and 22.

Table 4. Binding affinities of compounds 36 and 37 on the hGnRH receptor[7]

Compd	K_i (nM) human
36	270
37	64

With these results, subsequent SAR studies were focused on replacing the 6-carboxylates with carboxamides. As shown in Table 3, 3-pentyl carboxamide 26 was substantially less potent than its 3-pentyl ester analogue 22. However, cyclopentyl analogue 27 was 2-fold more potent than 26. While expanding the ring from cyclopentyl to cyclohexyl (29) caused a decrease in potency, a linear butyl carboxamide 28 was equally potent as the cyclopentyl analogue 27. Further extension of the butyl to hexyl (30) provided a 3-fold increase in binding affinity. However, insertion of an oxygen atom in the butyl chain for reducing lipophilicity also reduced potency (31). The preferred side chain from this limited optimization study was a 3-phenylpropyl carboxamide group (32) which yielded another 3-fold increase in potency in comparison with its hexyl analogue 30. Unlike the 3-pentyl ester, incorporation of carboxamide at position-6 required the presence of the *N*-methyl-*N*-[2-(2-pyridyl)]ethylamine on 1-methyl position for high potency again. For example, 33 decreased almost 17-fold in potency only due to switch of side chain of the basic amine from a 2-(2-pyridyl)ethyl group to a benzyl group. Similar results were observed in 34 and 35. Compared to 28, the potencies of these two compounds were dramatically reduced simply because benzyl and 2-diethylaminoethyl instead of 2-(2-pyridyl)ethyl were substituted on the basic amine.

Since position 6 was well tolerated for modification, a limited study was undertaken to explore the use of more stable heterocyclic groups to replace the esters and amides. The resulting compounds 36 and 37 (Table 4) had K_i values of 270 and 64 nM, respectively, which were comparable to the potency of the corresponding esters and amides. These results promoted us to perform more modifications at position 6 using aryl groups and the results will be presented elsewhere in the near future.

In order to demonstrate functional antagonism, selected compounds were evaluated for their ability to inhibit GnRH stimulated calcium flux.[8] As shown in Figure 1, compounds 20 and 22 at a concentration of 1 μM were able to completely block Ca^{++} flux stimulated by 10 nM GnRH. No indication of stimulatory activity for these or other compounds tested was observed.

In conclusion, we have developed a novel and efficient two-step synthesis for 2-arylpyrrolo[1,2-*a*]pyrimid-7-ones. Further modifications of these structures led to the discovery of a series of highly potent hGnRH receptor antagonists. SAR study of these antagonists indicated that hydrogen is more preferred substituent than a cyano group at position 3 of this bicyclic core structure. Position 6 was amenable to substitution by a variety of groups without compromising the binding affinity to the hGnRH receptor.

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6. Human GnRH receptor was stably expressed in HEK293 cells and a 96-well filtration assay was used.
7. On each assay plate, a standard antagonist of comparable affinity to those being tested was included as a control for plate-to-plate variability. Overall, K_i values were highly reproducible with an average standard deviation of 45% for replicate K_i determinations. Most of compounds reported here were assayed 2–8 times.
8. HEK293 cells stably expressing the hGnRH receptor were loaded with the calcium sensitive dye Indo-1 then pre-incubated with compound 20, 22 or vehicle control for 1 min prior to stimulation with 10 nM GnRH. Calcium mobilization was measured by the change in fluorescence intensity ratio (490 nm/405 nm) following excitation at 350 nm.